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Colorful invasion in permissive Neotropical ecosystems: establishment of ornamental non-native poeciliids of the genera *Poecilia*/*Xiphophorus* (Cyprinodontiformes: Poeciliidae) and management alternatives

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Headwater creeks are environments susceptible to invasion by non-native fishes. We evaluated the reproduction of 22 populations of the non-native livebearers guppy *Poecilia reticulata*, black molly *Poecilia sphenops*, Yucatan molly *Poecilia velifera*, green swordtail *Xiphophorus hellerii*, southern platyfish *Xiphophorus maculatus*, and variable platyfish *Xiphophorus variatus* during an annual cycle in five headwater creeks located in the largest South American ornamental aquaculture center, Paraíba do Sul River basin, southeastern Brazil. With few exceptions, females of most species were found reproducing (stages 2, 3, 4) all year round in the creeks and gravid females of all species showed small sizes indicating stunting. Juveniles were frequent in all sites. The fecundity of the six poeciliids was always low in all periods. The sex ratio was biased for females in most species, both bimonthly as for the whole period. Water temperature, water level and rainfall were not significantly correlated with reproduction in any species. Therefore, most populations appeared well established. The pertinence of different management actions, such as devices to prevent fish escape, eradication with rotenone and research about negative effects on native species, is discussed in the light of current aquaculture practices in the region.

Keywords: Aquaculture, Invasive species, Livebearers, Reproduction, Stream.

Riachos de cabeceira são ambientes susceptíveis à invasão por peixes não-nativos. Neste trabalho, avaliou-se a reprodução de 22 populações dos poecilídeos não-nativos guppy *Poecilia reticulata*, molinésia preta *Poecilia sphenops*, molinésia *Poecilia velifera*, espadinha *Xiphophorus hellerii*, plati *Xiphophorus maculatus* e plati variado *Xiphophorus variatus* durante diferentes anos em cinco riachos de cabeceira localizados no maior polo de piscicultura ornamental da América do Sul, bacia do rio Paraíba do Sul, sudeste do Brasil. Foram encontradas fêmeas da maioria das espécies em reprodução (estágios 2, 3, 4), durante todos os anos nos riachos e fêmeas grávidas de todas as espécies apresentaram pequeno tamanho indicando nanismo. Juvenis de todas as espécies foram frequentes em todos os locais. A fecundidade das seis espécies sempre foi baixa em todos os períodos. Para os períodos bimestrais e totais, encontrou-se mais fêmeas que machos na maioria das espécies. Temperatura e nível de água dos riachos, bem como precipitação pluviométrica não foram correlacionadas com a reprodução. Todas as populações estão estabelecidas nos locais e ações de gerenciamento como dispositivos para impedir a fuga de peixes, erradicação com rotenona e pesquisa científica sobre os efeitos negativos nas espécies nativas são discutidas em relação às práticas de piscicultura na região.

Palavras-chave: Aquicultura, Córrego, Espécies invasoras, Ovovivíparos, Reprodução.

Introduction

Headwater creeks occur in all landscapes around the globe, and may compose almost 80% of total stream length in many drainage networks (Sidle *et al*., 2000). They offer an enormous array of habitats for microbial, plant, and animal life, and are sensitive areas for the conservation of aquatic biodiversity due to their low species richness and high degree of endemism (Abell *et al*., 2007). These characteristics define them as permissive environments, *i.e.* susceptible to the establishment of non-native species (Courtenay *et al*., 1974), and are among the most threatened systems in the planet (Dudgeon *et al*., 2006).

In South America and Brazil, native communities in headwater creeks are suffering changes in their original structure due to changes in riparian vegetation, siltation, pollution, small dams, recreational activities, cattle ranching and non-native fish introductions resulting from human involvement (Bizerril, Lima, 2001; Castellani, Barrella, 2006; Casatti *et al*., 2009; Magalhães, Jacobi, 2013a).

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Although more than 50 non-native fish species are currently found in Brazilian headwater creeks (Bizerril, Lima, 2001; Magalhães, Jacobi, 2008), a group of seven poeciliids deserve special attention as a threat to native communities due to negative impacts such as changes in the structure of the native fish assemblages and biotic homogenization: the guppy *Poecilia reticulata* Peters, 1859, sailfin molly *P. latipinna* (Lesueur 1821), black molly *P. sphenops* Valenciennes in Cuvier, Valenciennes, 1846, Yucatan molly *P. velifera* (Regan 1914), green swordtail *Xiphophorus hellerii* Heckel 1848, southern platyfish *X. maculatus* (Günther 1866), and variable platyfish *X. variatus* (Meek 1904) (Alves *et al*., 2007; Magalhães, Jacobi, 2008; Magalhães *et al*., 2011). Along with cyprinids, cichlids and osphronemids, these poeciliids are the ornamental fishes most sought by the Brazilian aquarium trade (Coe *et al*., 2011; Magalhães, Jacobi, 2013b; Assis *et al*., 2014; Garcia *et al*., 2014). They all have been introduced into headwater creeks of the southeastern region mainly as by-products of aquaculture-related activities (Bizerril, Lima, 2001; Castellani, Barrella, 2006; Alves *et al*., 2007; Magalhães, Jacobi, 2008). Poeciliidae, however, has been identified as one of the top invasive freshwater fish families and is linked to reduction in zooplankton, damselfly/dragonfly populations, decline and extinction of native fishes and also to negatively impact native amphibians by preying on amphibian eggs and larval stages in several regions of the world (Stockwell, Henkanaththegedara, 2011). Because they are livebearers, their members have an invasive advantage over species of other popular non-native families (Milton, Arthington, 1983; Deacon *et al*., 2011).

It is the initial and final stages of biological invasions that are usually the target of most studies in this field, but intermediate stages, involving the dynamics of reproduction, should deserve equal attention (Williamson, 2000). Reproductive characteristics have substantial effects on a species invasion success, and a thorough knowledge of the reproductive biology of poeciliids outside their natural ranges is a fundamental prerequisite to assess the extent of their establishment and potential ecological impacts on native communities of headwater creeks.

Thus, our goal was to evaluate the reproductive status (*i.e.* establishment) of 22 populations of non-native aquarium poeciliids in five headwater Brazilian creeks, determine their response to local abiotic factors (water temperature/level, rainfall), discuss their impacts and suggest management measures to protect these peculiar ecosystems.

Material and Methods

Study area. The Muriaé Ornamental Aquaculture Center (MOAC) was founded in 1979 at southeast of Minas Gerais State, Brazil. MOAC is the largest ornamental aquaculture area in South America, comprising 13 municipalities, two villages and is currently home to 350 fish farms, 4,500 fish ponds (Magalhães, Jacobi, 2008). This area provides 70% of the Brazilian aquarium fishes demand (Cardoso, Igarashi, 2009). The poeciliids *P. reticulata*, *P. sphenops*, *X. hellerii* and *X. maculatus* are among the 20 most cultured species, with a combined annual production of almost 3,000,000 individuals raised in monoculture ponds (Cardoso *et al*., 2012).

Field collections. The vegetation cover in the region consists of Atlantic Forest remnants (*i.e.* mesophyllous semideciduous forest) interspersed with grazing and monoculture cultivations initiated since the 1940´s. Five headwater creeks (first-order, *sensu* Strahler, 1957) were surveyed, all belonging to the Paraíba do Sul River basin, one of the main South American watersheds (Albert, Reis, 2011). These creeks run across a region with hundreds of culture ponds surrounding the major municipalities of the aquaculture center. They are slow-flowing warmwater habitats, have clean water; with an average width of 137 cm, sandy/muddy bottoms devoid of aquatic macrophytes, their mud banks do not have riparian vegetation, are covered by non-native grasses *Brachiaria* spp. (syn. *Urochloa* spp.) due to cattle ranching, and are subjected to flash floods in the rainy season (Magalhães, Jacobi, 2013a). Their native ichthyofauna is composed of a few small characids, cichlids, loricariids, trichomycterids, and poeciliids like the spotsided livebearer *Poecilia vivipara* Bloch & Schneider 1801 and the endemic dusky millions fish *Phalloceros* cf. *leptokeras* Lucinda 2008 (Vieira, Rodrigues, 2010). In all sites of this study, the 369 production ponds are drained every 45 days, which represents almost 3,000 pond drainage events per year. This practice creates a massive propagule pressure, given that ponds have no screens on the effluent pipes to prevent escapes.

Fish were collected every two months, from January to December, in different years in each creek: Boa Vista (21º01'15"S; 42º21'20"W) in 2003; Pinheiros (20º53'26"S; 42º20'33"W) in 2004; Santo Antônio (20º53'14"S; 42º17'26"W) in 2005; Chato (20º53'28"S; 42º22'36"W) and Gavião (20º54'30"S; 42º24'38"W) in 2006, southeastern Brazil (Fig. 1). Juveniles and adults were collected by the same person from margins and channel beds, with a rectangular sieve measuring 95 cm long × 25 cm high and 0.3 mm mesh. The sieve was cast 50 times along 100 m-long transects during 2 h in each creek at daytime. The fishes (Tab. 1) were euthanized on an ice slurry, a method approved for field work by IACUC (2002), sorted by site collection, packed in plastic bags, fixed in 10% formalin, and subsequently transferred to 70% alcohol. Voucher specimens used in this study are deposited under their respective catalogue numbers in the Museu de Ciências e Tecnologia da Pontifícia Universidade Católica do Rio Grande do Sul (MCP) and in the Universidade Federal de Sergipe fish collection (CIUFS). In the laboratory, the species were measured (standard length SL - given in cm) and sexed as males if there was any evidence of a gonopodium, as juveniles if smaller than the smallest male in each sample, and as females in the absence of a gonopodium but larger than the smallest male (Gkenas *et al*., 2012).

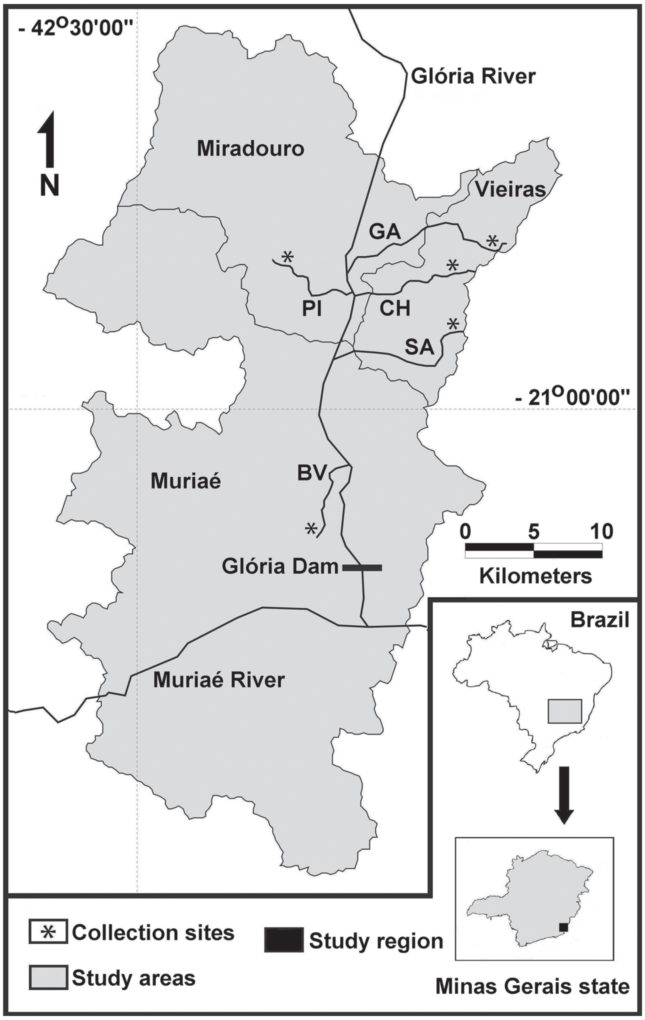

Fig. 1. Sampling locations of *Poecilia* and *Xiphophorus* species. Municipalities: Muriaé, Miradouro, Vieiras. Headwater creeks: BV Boa Vista, PI Pinheiros, SA Santo Antônio, CH Chato, GA Gavião (modified from Magalhães, Jacobi, 2013a).

Developmental stages. We adapted the methods of Winemiller (1993) (macroscopic) and Haynes (1995) (mesoscopic) to determine the developmental stages of adult females. We classified as non-gravid females those without a conspicuously enlarged ventral region, absence of eggs, embryos, or gravid spot near the base of the anal fin. On the other hand, females with an enlarged belly, presence of gravid spot (not seen in females of *P. sphenops* and *P. velifera* due to their solid black and marble colours respectively), eggs, or embryos seen with the naked eye were classified as gravid females. On a mesoscopic scale, developmental stages were classified as follows: Non-gravid (stage 1 - ovarian tissue without ovum and/or embryos), Yolked ova (stage 2 - ovum in the process of yolking, yellow in guppy, green swordtail, southern platyfish, variable platyfish and orange in black molly, Yucatan molly; oil droplets, if present, unevenly distributed), Eyed embryos (stage 3 - eyes pigmented, head enlarged compared to trunk, caudal and pectoral fin buds present, dorsal pigmentation initiated in all species), Mature embryos (stage 4 - yolk sac mostly or completely absorbed, pectoral fins elongate, scales present, embryo resembling a small adult). After determining the developmental stages, the bimonthly absolute and relative frequencies of females were calculated.

Population structure, fecundity and sex ratio. The adult-to-juvenile absolute and relative frequencies and ratio (Pope *et al*., 2010) were calculated from the total number of females, males and juveniles of all poeciliid species. We estimated the bimonthly and total mean values of fecundity (embryos in stages 3 and 4) of all species in the creeks. Only embryos were used as a measure of fecundity because in the ova development is not readily discernible with a dissecting microscope and it is not known whether they have been fertilized (Haynes, 1995). The sex ratio (Pope *et al*., 2010) was calculated from the bimonthly and total number of females and males of the six species collected in the five creeks.

Tab. 1. Number of ornamental non-native poeciliids used to study developmental stages, adult (♀, ♂)/juvenile (J) frequency, fecundity and sex ratios in 2003, 2004, 2005 and 2006.

Species Voucher specimens	Boa Vista			Pinheiros			Santo Antônio			Chato			Gavião		
	♀	♂	J	♀	♂	J	♀	♂	J	♀	♂	J	♀	♂	J
Poecilia reticulata	87	24	442	103	104	531	514	192	2,258	601	401	3,427	590	317	1,270
Voucher number		CIUFS 2601			CIUFS 2604			CIUFS 2597			CIUFS 2608			CIUFS 2613	
Poecilia sphenops		-			-		168	91	341	242	143	175		-	
Voucher number		-			-			CIUFS 2600			CIUFS 2611			-	
Poecilia velifera		-			-		97	53	243		-			-	
Voucher number		-			-			MCP 39003			-			-	
Xiphophorus hellerii	140	56	203	145	43	225	208	108	401	363	195	697	536	76	530
Voucher number		CIUFS 2603			CIUFS 2606			CIUFS 2599			CIUFS 2610			CIUFS 2614	
Xiphophorus maculatus	80	77	151	161	84	138	128	132	110	261	156	120	355	207	133
Voucher number		CIUFS 2602			CIUFS 2605			CIUFS 2598			CIUFS 2609			CIUFS 2615	
Xiphophorus variatus		-		119	103	351	278	75	269	284	104	494	538	265	1,002
Voucher number		-			CIUFS 2607			MCP 39004			CIUFS 2612			CIUFS 2616	

Correlation between reproduction and abiotic factors. Abiotic data were collected every two months, namely water temperature, level, and rainfall. The first two were obtained at the times of the fish sampling in each creek. We measured water temperature in the field with a mercury-in-glass thermometer to the scale 0º to 50ºC, and water level using a pole marked every 10 cm. Rainfall data are from the database of the INPE (2006) located in the municipalities of Muriaé, Miradouro, and Vieiras. Following Andrade, Braga (2005), we grouped the bimonthly relative frequencies of gravid females (stages 2, 3, and 4), which were then correlated with the average bimonthly water temperature, water level and rainfall.

Statistical analyses. Chi-square (χ^2 test) goodness of fit tests were conducted to determine the differences in proportions between juveniles-to-adults and sexes, and the Spearman`s rank correlation to test a relationship between female reproduction and abiotic factors (Sokal, Rohlf, 1995). Differences were significant when $P < 0.05$. All statistical analyses were performed using PAST-Paleontological Statistics (version 1.91) software (Hammer *et al*., 2009).

Results

Developmental stages and early maturity. All poeciliid species showed a long reproductive period, varying from eight to 12 months (Figs. 2-6). Females were found in several developmental stages during almost all the sampling period in the creeks. The exceptions were *P. reticulata* in Boa Vista Creek where females showed no reproductive activity in March/April 2003 (Fig. 2), and *P. velifera* in Santo Antônio Creek, which were not collected from March to June 2005 (Fig. 4). Although all species showed reproductive activity, the proportion of non-gravid females differed among species. In particular, *P. sphenops*, *P. velifera*, and *X. hellerii* showed low percentages of gravid females in most bimesters, while *X. variatus* was rarely under 40%. These proportions also differed among creeks, with Santo Antônio showing a higher reproductive activity (Fig. 4), and Gavião with less (Fig. 6). In Boa Vista Creek, the smallest gravid female of *P. reticulata* measured 1.8 cm SL, *X. hellerii* 3.3 cm SL and *X. maculatus* measured 2.8 cm SL. In Pinheiros Creek, *P. reticulata* measured 1.9 cm SL, *X. hellerii* 3.5 cm SL, *X. maculatus* 2.8 cm SL and *X. variatus* measured 2.9 cm SL. In Santo Antônio Creek, *P. reticulata* 2.1 cm SL, *P. sphenops* 3.0 cm, *P. velifera* 3.0 cm SL, *X. hellerii* 3.2 cm, *X. maculatus* 2.6 cm SL and *X. variatus* measured 2.7 cm SL. In Chato Creek, *P. reticulata* female measured 2.1 cm SL, *P. sphenops* 2.5 cm SL, *X. hellerii* 3.2 cm SL, *X. maculatus* 2.5 cm SL, *X. variatus* 2.4 cm SL, and in the Gavião Creek, the smallest gravid female of *P. reticulata* measured 2.0 cm SL, *X. hellerii* 2.5 cm SL, *X. maculatus* 1.9 cm SL and *X. variatus* measured 2.2 cm SL.

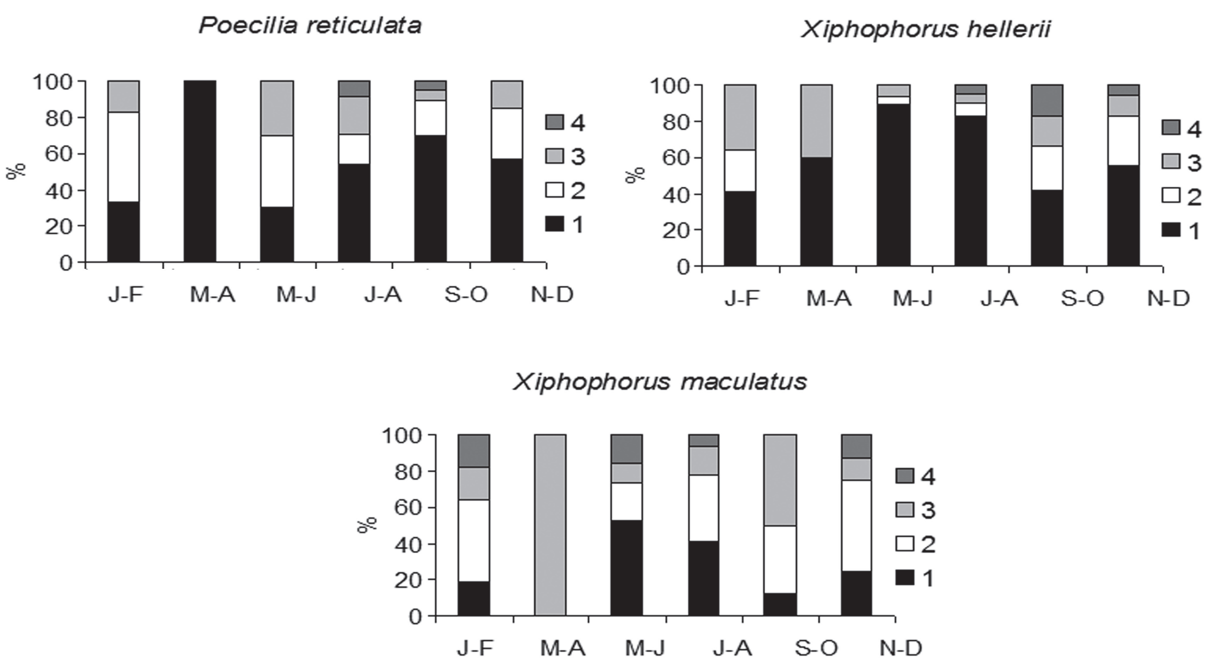

Fig. 2. Relative bimonthly frequency of developmental stages in ornamental non-native poeciliid females collected in Boa Vista Creek, 2003. Developmental stages: 1 Non-gravid, 2 Yolked ova, 3 Eyed embryos, 4 Mature embryos.

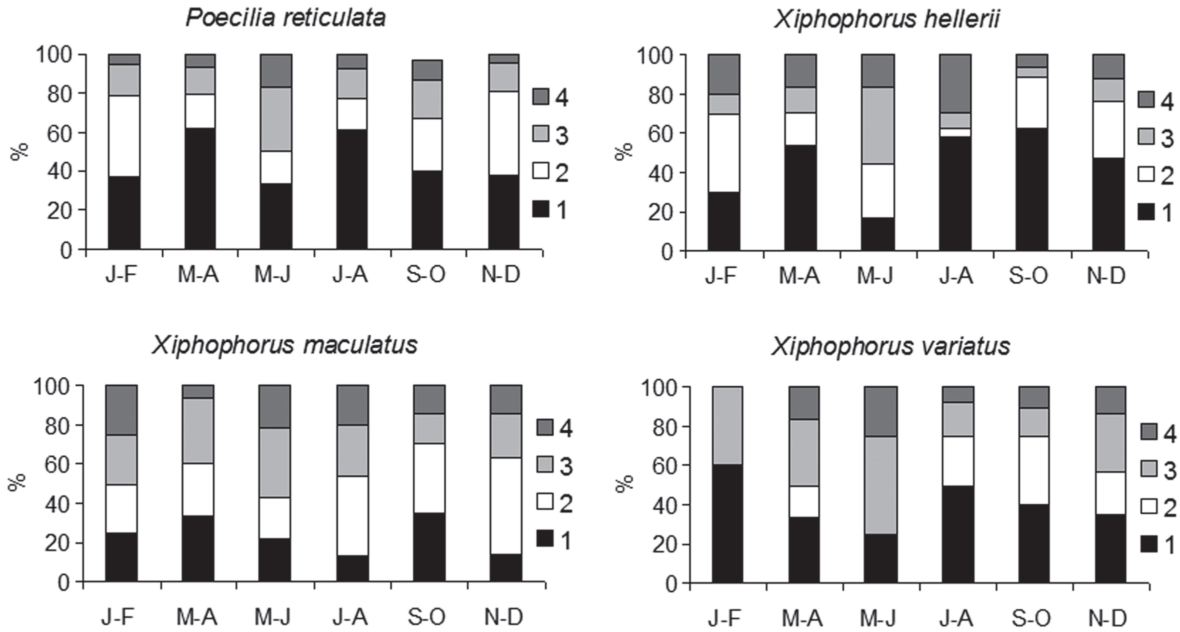

Fig. 3. Relative bimonthly frequency of developmental stages in ornamental non-native poeciliid females collected in Pinheiros Creek, 2004. Developmental stages: 1 Non-gravid, 2 Yolked ova, 3 Eyed embryos, 4 Mature embryos.

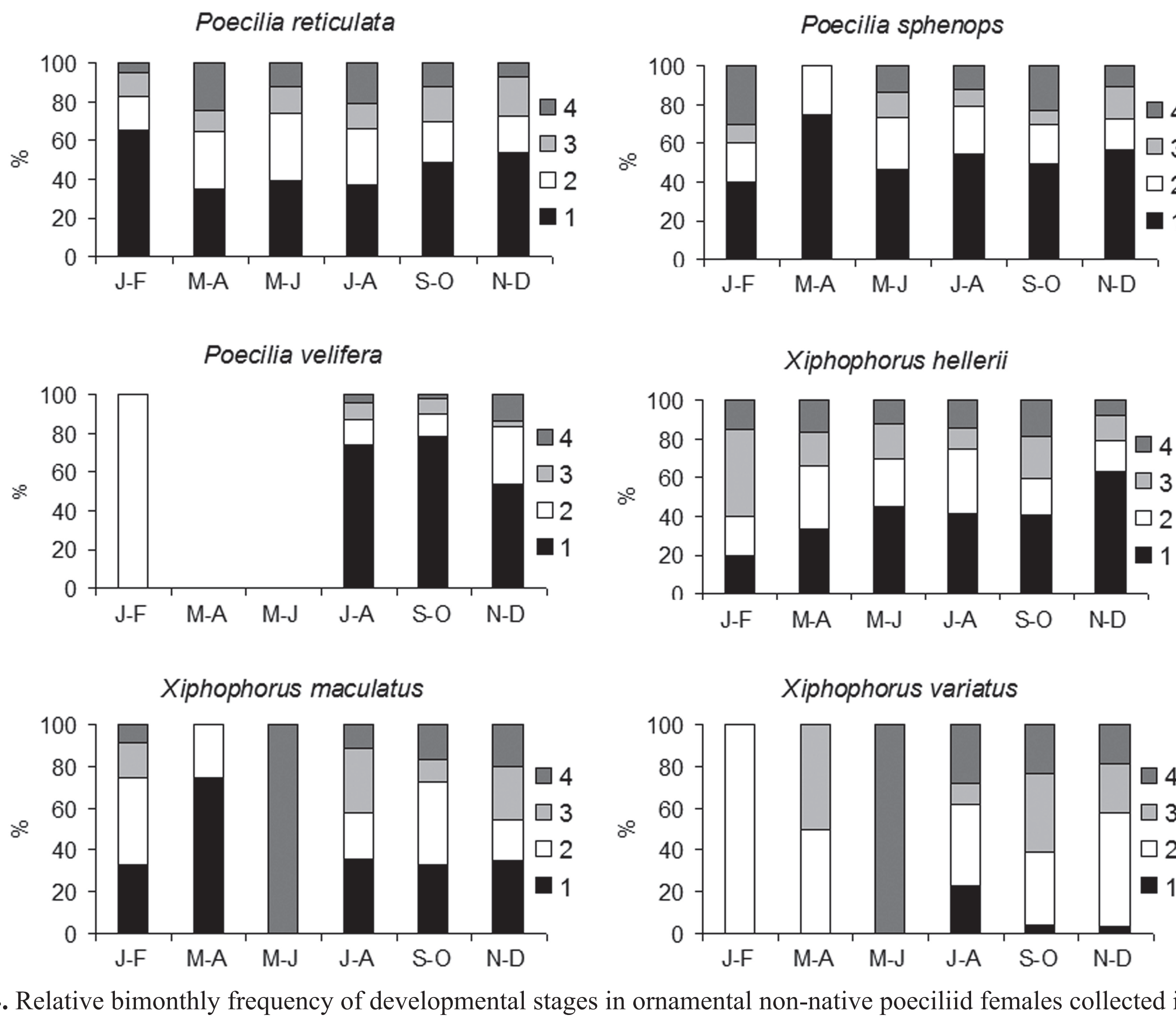

Fig. 4. Relative bimonthly frequency of developmental stages in ornamental non-native poeciliid females collected in Santo Antônio Creek, 2005. Developmental stages: 1 Non-gravid, 2 Yolked ova, 3 Eyed embryos, 4 Mature embryos.

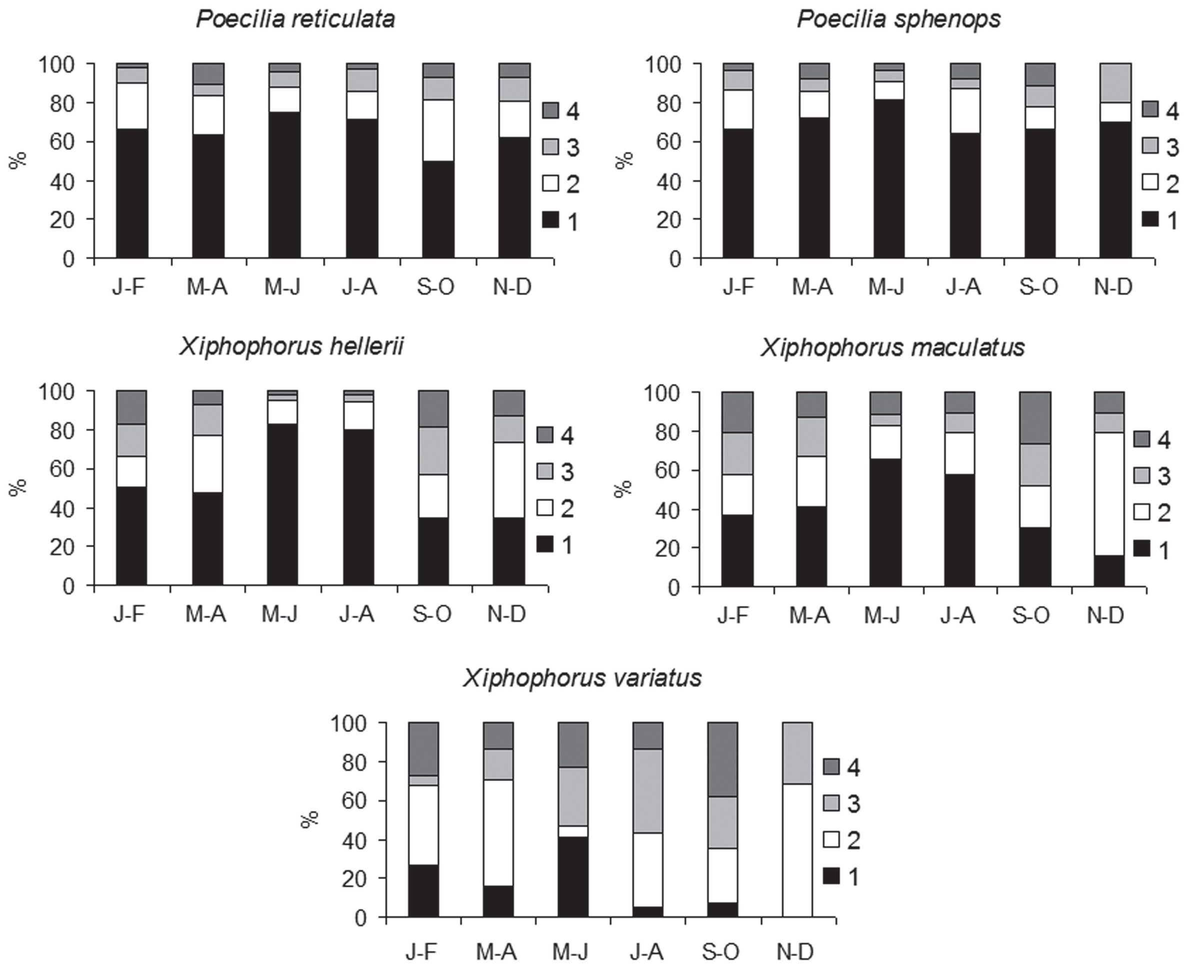

Fig. 5. Relative bimonthly frequency of developmental stages in ornamental non-native poeciliid females collected in Chato Creek, 2006. Developmental stages: 1 Non-gravid, 2 Yolked ova, 3 Eyed embryos, 4 Mature embryos.

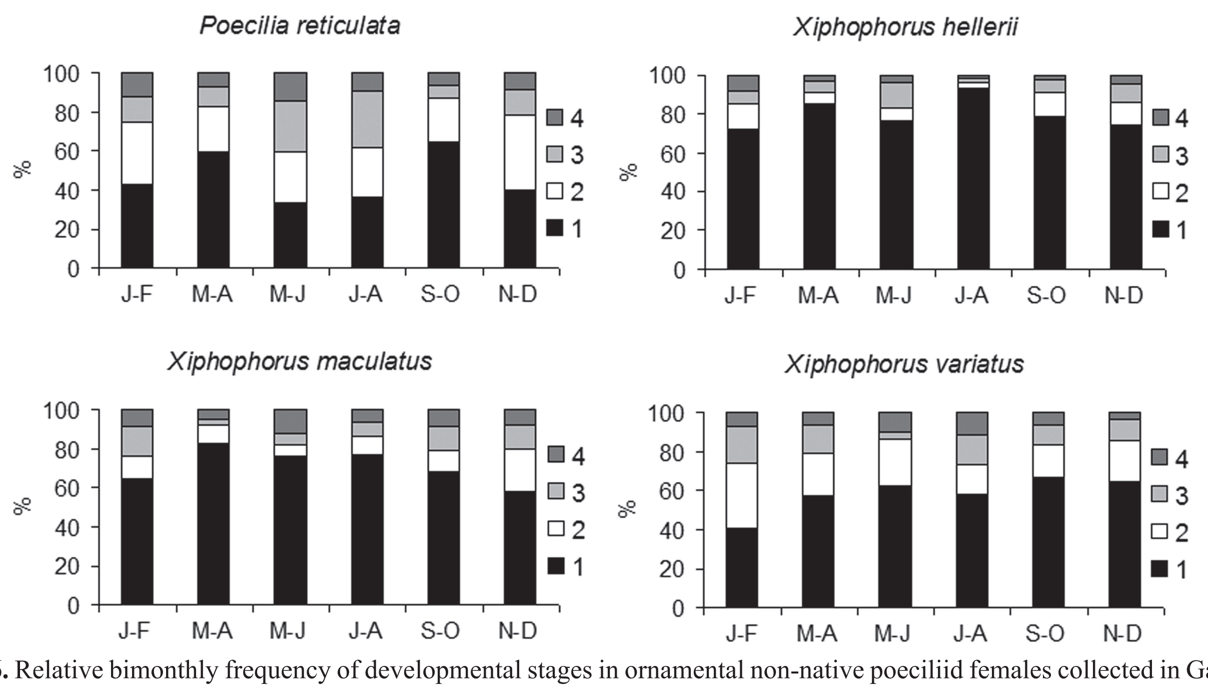

Fig. 6. Relative bimonthly frequency of developmental stages in ornamental non-native poeciliid females collected in Gavião Creek, 2006. Developmental stages: 1 Non-gravid, 2 Yolked ova, 3 Eyed embryos, 4 Mature embryos.

Population structure, fecundity and sex ratio. Non-native juvenile poeciliids of undetermined sex were caught in all sites. Non-native juveniles had a total number of fish captured of 13,511 specimens and a proportion of 95.17%. These values represent significant amounts and proportion when compared with the low number (686) and proportion of native juveniles (4.83%). For the total period, the number of non-native juvenile poeciliids was higher than adult females and males in 12 populations (54.54%). Conversely, the number of juveniles was lower than adult females and males in seven populations (31.82%) captured in Pinheiros (*X. maculatus* 63.97%; χ^2=29.9; d.f.=1; P < 0.05), Santo Antônio (*X. maculatus* 70,27%; χ^2=60.8; d.f.=1; P < 0.05, *X. variatus* 56.75%; χ^2=11.34; d.f.=1; P < 0.05), Chato (*P. sphenops* 68.75%; χ^2=78.75; d.f.=1; P < 0.05, *X. maculatus* 77.65%; χ^2=164.26; d.f.=1; P < 0.05) and Gavião (*X. hellerii* 53.59%; χ^2=5.88; d.f.=1; P < 0.05, *X. maculatus* 80.86%; χ^2=264.8; d.f.=1; P < 0.05) creeks (Tab. 2).

The mean fecundity of the six species was low in all bimesters and also for the total period in comparison with other introduced populations. The lowest fecundities, below seven embryos per brood, were observed in *P. reticulata* in all creeks (4.81±0.62, 6.05±1.59, 5.93±0.90, 5.25±1.19, 6.14±1.61), *X. maculatus* in Santo Antônio and Chato creeks respectively (6.59±1.98, 6.23±0.44), and *X. variatus* in Gavião Creek (6.72±1.60). The highest values corresponded to *X. helleri* in Pinheiros Creek, with more than 10 embryos per brood (Fig. 7).

Overall, females were significantly more abundant than males in all sites. In Boa Vista Creek, females were more frequent than males in guppy (χ^2=34.94; 3.5:1; d.f.=1; P < 0.05) and green swordtail (χ^2=36.00; 2.5:1; d.f.=1; P < 0.05). We found no significant differences in sex ratio of southern platyfish (χ^2=0.00; 1:1; d.f.=1; P < 0.05). In Pinheiros Creek, we found more females than males in *X. hellerii* (χ^2=55.34; 3.40:1; d.f.=1; P < 0.05) and *X. maculatus* (χ^2=24.20; 1.90:1; d.f.=1; P < 0.05), whereas the sex ratio of *P. reticulata* and variable platyfish was almost identical (χ^2=0.00; 0.99:1; d.f.=1; P < 0.05, χ^2=1.16; 1.15:1; d.f.=1; P < 0.05). In Santo Antônio Creek, females were significantly more frequent than males in *P. reticulata*, *P. sphenops*, *P. velifera*, *X. hellerii,* and *X. variatus*. On the other hand, males and females had the same frequencies in *X. maculatus* (χ^2=0.06; 0.97:1; d.f.=1; P < 0.05). In Chato Creek, females were significantly more frequent than males in all five species. In Gavião Creek, females of *P. reticulata*, *X. hellerii*, *X. maculatus*, and *X. variatus* were significantly more frequent than males (χ^2=82.18; 1.86:1; d.f.=1; P < 0.05, χ^2=345.75; 7.05:1; d.f.=1; P < 0.05, χ^2=38.98; 1.71:1; d.f.=1; P < 0.05, χ^2=92.82; 2.03:1; d.f.=1; P < 0.05) (Tab. 3).

Correlation between reproduction and abiotic factors. Water temperature, water level, and rainfall were not significantly correlated with reproduction of females in any of the six species or creeks (Tab. 4).

Tab. 2. Total absolute/relative frequencies and adult-to-juvenile ratio of ornamental non-native poeciliid species collected in the creeks in 2003, 2004, 2005 and 2006. Adult (♀+♂), juvenile (J). * Indicates the significant values for adult-to-juvenile ratio (Chi-square, P < 0.05, $\chi^2_{0.05}$ = 3.841, d.f. = 1).

Species	Boa Vista			Pinheiros			Santo Antônio			Chato			Gavião		
%/Adult-juvenile ratio	♀	♂	J	♀	♂	J	♀	♂	J	♀	♂	J	♀	♂	J
Poecilia reticulata	87	24	442	103	104	531	514	192	2,258	601	401	3,427	590	317	1,270
% (♀+♂/J)	20.07		79.93	33.05		71.95	23.82		76.18	22.62		77.38	41.66		58.34
Chi-square (♀+♂/J)		198.12*			142.24*			812.66*			1.327.76*			60.52*	
Poecilia sphenops		-			-		168	91	341	242	143	175		-	
% (♀+♂/J)		-			-		43.17		58.83	68.75		31.25		-	
Chi-square (♀+♂/J)		-			-			11.2*			78.75*			-	
Poecilia velifera		-			-		97	53	243		-			-	
% (♀+♂/J)		-			-		38.17		61.83		-			-	
Chi-square (♀+♂/J)		-			-			22.00*			-			-	
Xiphophorus hellerii	140	56	203	145	43	225	208	108	401	363	195	697	536	76	530
% (♀+♂/J)	32.55		33.72	45.52		54.48	44.07		55.93	44.46		55.54	53.59		46.41
Chi-square (♀+♂/J)		0.12			3.32			10.08*			15.4*			5.88*	
Xiphophorus maculatus	80	77	151	161	84	138	128	132	110	261	156	120	355	207	133
% (♀+♂/J)	50.97		49.03	63.97		36.03	70.27		29.73	77.65		22.35	80.86		19.14
Chi-square (♀+♂/J)		0.12			29.9*			60.8*			164.26*			264.8*	
Xiphophorus variatus		-		119	103	351	278	75	269	284	104	494	538	265	1,002
% (♀+♂/J)		-		38.74		61.26	56.75		43.25	43.99		56.01	44.49		55.51
Chi-square (♀+♂/J)		-			23.94*			11.34*			12.74*			21.94*	

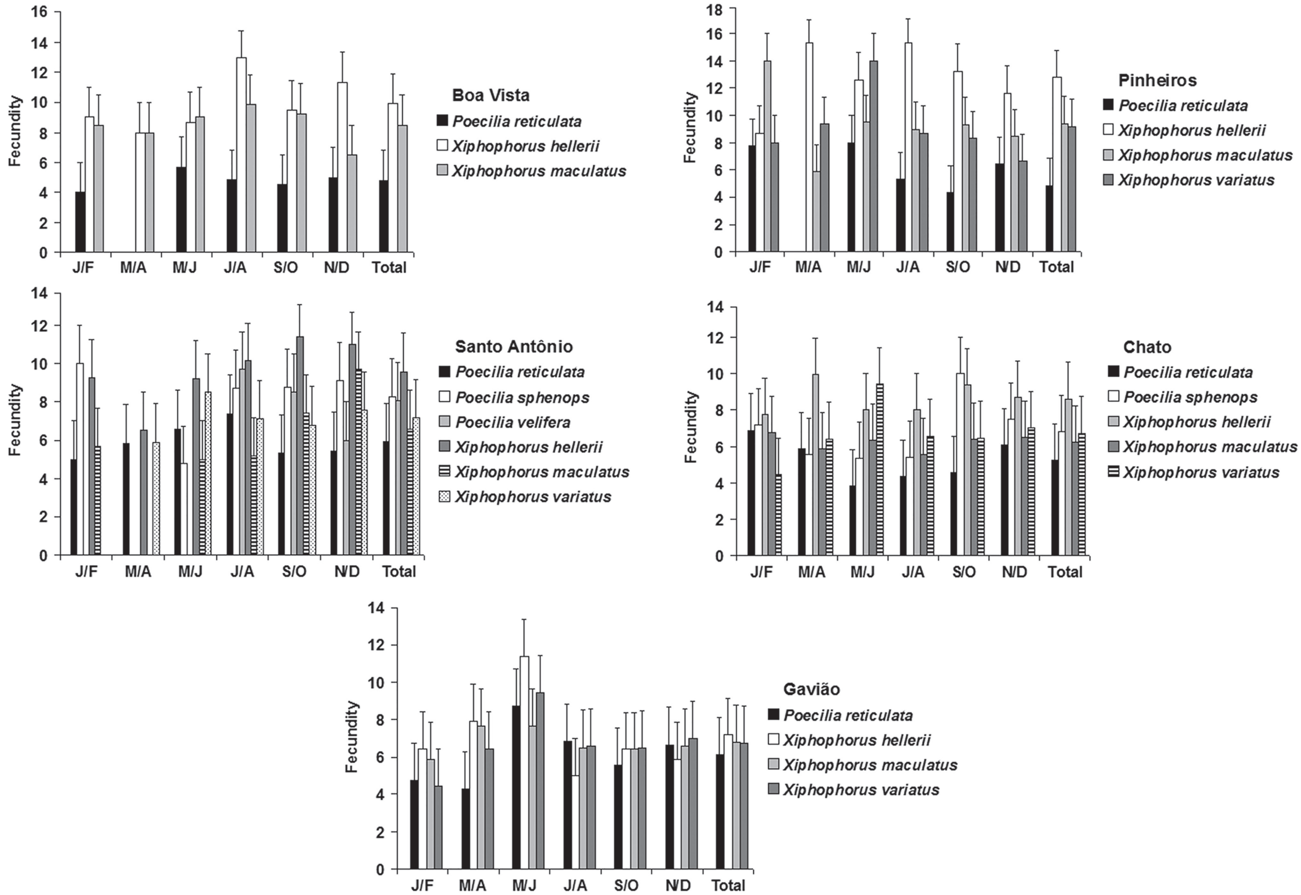

Fig. 7. Bimonthly and total fecundity (number of embryos per brood) of ornamental non-native poeciliid species collected in the creeks in 2003, 2004, 2005 and 2006.

Tab. 3. Bimonthly and total sex ratio of ornamental non-native poeciliid species collected in the creeks in 2003, 2004, 2005 and 2006. * Indicates the significant values for sex ratio (Chi-square, $P < 0.05$, $\chi^2_{0.05} = 3.841$, d.f. = 1).

Species/Sex ratio	Boa Vista	Pinheiros	Santo Antônio	Chato	Gavião
Poecilia reticulata					
January-February	5.40*	3.58	66.56*	5.36*	13.30*
March-April	1.00	1.48	23.52*	4.26*	40.82*
May-June	9.00*	0.00	13.12*	11.02*	12.48*
July-August	9.32*	2.32	32.50*	4.78*	9.26*
September-October	9.00*	0.14	12.94*	10.32*	4.94*
November-December	1.60	1.00	16.82*	5.90*	11.74*
Total/SR ♀: ♂	34.94*/3.5	0.00/0.99	146.86*/2.68	39.92*/1.50	82.18*/1.86
Poecilia sphenops					
January-February	-	-	7.36*	10.22*	-
March-April	-	-	0.00	11.60*	-
May-June	-	-	9.94*	1.14	-
July-August	-	-	12.30*	3.58	-
September-October	-	-	19.46*	1.14	-
November-December	-	-	1.42	0.06	-
Total/SR ♀: ♂	-	-	22.88*/1.85	25.46*/1.69	-
Poecilia velifera					
January-February	-	-	2.00	-	-
March-April	-	-	0.00	-	-
May-June	-	-	0.00	-	-
July-August	-	-	11.58*	-	-
September-October	-	-	4.90*	-	-

Tab. 3. (continued)

Species/Sex ratio	Boa Vista	Pinheiros	Santo Antônio	Chato	Gavião
November-December	-	-	0.66	-	-
Total/SR ♀: ♂	-	-	12.90*/1.83	-	-
Xiphophorus hellerii					
January-February	2.46	3.76	10.66*	10.34*	47.12*
March-April	2.66	9.32*	1.00	28.60*	89.72*
May-June	28.58*	0.80	13.50*	8.68*	35.28*
July-August	11.36*	14.28*	1.00	7.18*	66.22*
September-October	0.42	22.50*	5.06*	2.78	69.20*
November-December	0.50	11.76*	5.36*	0.02	45.74*
Total/SR ♀: ♂	36.00*/2.5	55.34*/3.40	31.64*/1.93	50.58*/1.86	345.75*/7.05
Xiphophorus maculatus					
January-February	0.88	0.66	2.88	0.14	3.62
March-April	0.34	12.24*	0.00	22.74*	28.34*
May-June	0.58	7.12*	1.80	1.04	5.26*
July-August	0.03	3.86*	0.52	0.36	3.00
September-October	0.22	0.68	0.00	6.12*	0.74
November-December	1.34	15.52*	0.02	0.02	3.04
Total/SR ♀: ♂	0.00/1	24.20*/1.90	0.06/0.97	26.44*/1.67	38.98*/1.71
Xiphophorus variatus					
January-February	-	0.00	1.00	5.46*	41.68*
March-April	-	0.06	4.00*	32.58*	4.64*
May-June	-	0.00	0.34	49.00*	11.52*
July-August	-	0.56	50.76*	34.10*	7.90*
September-October	-	2.36	58.78*	25.00*	16.46*
November-December	-	0.52	10.66*	11.92*	26.42*
Total/SR ♀: ♂	-	1.16/1.15	116.74*/3.71	83.50*/2.73	92.82*/2.03

Tab. 4. Spearman's rank correlation between ornamental non-native reproductive female poeciliids and abiotic variables in the creeks in 2003, 2004, 2005 and 2006.

Species/Variables	Boa Vista		Pinheiros		Santo Antônio		Chato		Gavião	
	r	P	r	P	r	P	r	P	r	P
Poecilia reticulata										
Gravid females × Water temperature	-0.883	0.07	0.116	0.83	-0.522	0.29	0.580	0.23	-0.551	0.26
Gravid females × Water level	-0.143	0.79	0.200	0.70	-0.600	0.21	0.371	0.47	-0.543	0.27
Gravid females × Rainfall	-0.257	0.62	0.200	0.70	-0.543	0.27	0.428	0.40	-0.428	0.40
Poecilia sphenops										
Gravid females × Water temperature	-	-	-	-	-0.232	0.66	0.116	0.83	-	-
Gravid females × Water level	-	-	-	-	0.143	0.79	-0.086	0.87	-	-
Gravid females × Rainfall	-	-	-	-	-0.086	0.87	-0.028	0.96	-	-
Poecilia velifera										
Gravid females × Water temperature	-	-	-	-	0.279	0.59	-	-	-	-
Gravid females × Water level	-	-	-	-	0.464	0.35	-	-	-	-
Gravid females × Rainfall	-	-	-	-	0.406	0.42	-	-	-	-
Xiphophorus hellerii										
Gravid females × Water temperature	0.530	0.28	-0.058	0.91	-0.116	0.83	0.580	0.23	0.783	0.07
Gravid females × Water level	0.771	0.07	0.314	0.54	0.257	0.62	0.428	0.40	0.600	0.21
Gravid females × Rainfall	0.829	0.06	0.314	0.54	-0.028	0.96	0.543	0.27	0.771	0.07
Xiphophorus maculatus										
Gravid females × Water temperature	0.765	0.08	-0.145	0.78	0.015	0.98	0.754	0.08	0.725	0.10
Gravid females × Water level	0.486	0.33	-0.314	0.54	0.087	0.87	0.486	0.33	0.314	0.54
Gravid females × Rainfall	0.543	0.27	-0.314	0.54	0	1	0.657	0.16	0.600	0.60
Xiphophorus variatus										
Gravid females × Water temperature	-	-	-0.696	0.12	0.031	0.21	-0.029	0.96	-0.029	0.96
Gravid females × Water level	-	-	-0.257	0.62	0.698	0.12	-0.200	0.70	0.486	0.33
Gravid females × Rainfall	-	-	-0.257	0.62	0.577	0.23	-0.028	0.57	0.314	0.54

Discussion

Population studies of non-native poeciliid species are rare in Brazil and this study provides a unique opportunity to evaluate the establishment of 22 populations of six ornamental non-native poeciliids in a same geographic region. We found females of *P. reticulata*, *P. sphenops*, *X. hellerii*, *X. maculatus*, and *X. variatus* in reproductive activity during the 12 months of the year in almost all creeks, with the exception of the guppy in Boa Vista and Yucatan molly in Santo Antônio creeks, that have reproductive periods of 10 and eight months respectively. Frequent reproduction over an extended breeding season is an opportunistic life-history strategy to overcome hydrologically unstable environments such as creeks and streams that are subject to flash floods, and provides a mechanism to maintain and/or reestablish the structure of population in poeciliid fishes (Winemiller, Rose, 1992). In this way, the continuous input of newborn individuals would assure a higher survival rate and therefore the maintenance of a viable population in a stochastic environment (Abilhoa *et al*., 2011). We also found gravid females of all non-native species with small sizes, which can indicate probable dwarfing or stunting. This phenomenon may be due to three reasons in the studied creeks: (i) intraspecific and interspecific competition due to overcrowding, (ii) low autochthonous food resources availability (typical of headwater habitats) and; (iii) increased survival rate due to the absence of large top predators (Olinger *et al*., 2016).

Juveniles of all six species were caught in all sites. The presence of juveniles is an indication of successful recruitment, and suggests that all 22 populations are established in these headwater creeks, which provide similar conditions to those of their places of origin, in tropical regions of Central and South America (Eschmeyer *et al*., 2016). All six poeciliid species produced small broods in comparison with larger broods recorded for these species introduced in other countries (Sa-nguansil, Lheknim, 2010; Froese, Pauly, 2016). According to Olinger *et al*. (2016), in upstream habitats (*i.e*. headwaters), female poeciliids have been shown to exhibit small broods and larger fingerlings when resources are scarce. Small broods may give these non-native livebearers a competitive advantage over native oviparous species, because fry are larger, feed at birth, grow more quickly and become predators´ faster (Rupp, 1996; Olinger *et al*., 2016).

A higher frequency of females (female-biased sex ratios), as was found in all creeks, appears to be common in other tropical regions. In poeciliids, males are more susceptible to mortality from a variety of sources, including differential predation (due to their bright colours), higher susceptibility to stressors, such as extreme temperatures, overcrowding, hypoxia, and also accelerated aging, since they invest their energy exclusively in mating behaviors (Snelson, 1989). Moreover, females do not depend on the constant presence of males to reproduce because they are able to retain active sperm in ovarian tissues for long periods and to bear successive broods without reimpregnation. These attributes of invasiveness allow them to colonize any environment by themselves (Deacon *et al*., 2011). The lack of correlation between abiotic factors was already expected, particularly considering the favourable temperature conditions throughout the years. This has been explained by ovoviviparity, a common reproductive strategy of the studied species (Snelson, 1989). Since eggs and embryos develop inside the female´s body, they are less influenced by several external conditions such as temperature variations, rain, diseases and predators attack (Oliveira *et al*., 2014).

The presence of reproductive adults and juveniles of non-native poeciliids in these creeks, besides characterizing establishment by recruitment, may lead to biotic homogenization, *i.e*. the establishment of the same non-native species at two or more locations, decreasing beta-diversity over time (Olden *et al*., 2011). This situation is already occurring in the area (Magalhães *et al.*, 2011), as well as the probable intra-specific hybridization between *X. maculatus* and *X. variatus* (A.L.B. Magalhães, pers. obs.). Likewise, it is also feasible that non-native *Poecilia* species hybridize with native congeners (*e.g. P. vivipara*), increasing the risk of native species extinction. According to Meyer *et al*. (2006) and Lampert, Schartl (2008), hybridization within *Xiphophorus* Heckel 1848 and *Poecilia* Bloch & Schneider 1801 is fairly common. Inter-specific competition for shelter, food and parturition sites could arise between non-native poeciliids and the livebearer endemic genus *Phalloceros* Eigenmann 1907 (Menezes *et al*., 2007). The nesting-guarding cichlids such as the chameleon cichlid *Australoheros muriae* Ottoni & Costa 2008, pearl cichlid *Geophagus obscurus* (Castelnau 1855) (= *Geophagus brasiliensis* Quoy & Gaimard 1824) and the loricariids *Hypostomus affinis* (Steindachner 1877) and *Neoplecostomus microps* (Steindachner 1877) will probably be unaffected by livebearers, but other native species that do not guard their eggs (*e.g.* lambari *Astyanax janeiroensis* Eigenmann 1908, lambari *Astyanax giton* Eigenmann 1908, catfishes *Trichomycterus* spp.) (Magalhães, Jacobi, 2013a), could be adversely affected. Species loss due to the influence of non-native poeciliids could be devastating because, according to Cruz *et al*. (2013), in systems with few native fish species such as headwater creeks, the loss of a single species might represent the deletion of an entire component of the food chain, compromising ecosystem functioning.

Although springheads and headwater creeks are legally considered permanent preservation areas they are often overlooked by conservation efforts in Brazil (Callisto *et al*., 2012). Given the current and future adverse ecological impacts derived from the presence of non-native poeciliids in these ecosystems, we propose management recommendations grouped into three categories: prevention, remediation and research. Preventing incursions of organisms outside of their native ranges is the most cost-effective way and public policies are needed to protect Brazil's headwater creeks from future introductions of new stocks of poeciliids and other non-native species. Ornamental aquaculture, as the main cause for poeciliid presence and spread throughout Brazil (Bizerril, Lima, 2001; Castellani, Barrella, 2006; Alves *et al*., 2007; Magalhães, Jacobi, 2008), should be controlled more carefully. We echo recommendations of Courtenay *et al*. (1974) in rerouting all effluent waters from fish rearing facilities through a dry well, and installation of adequate sand and gravel filter to allow passage of water but not livestock. We also suggest periodic inspection of ornamental fish farms to confirm the application of these measures. In addition, the Polluter Pays Principle should be enforced, since the introduction of non-native species is regarded as a form of environmental pollution, and environmental awareness campaigns on the negative effects of non-native species release into the wild should also be used, as proposed before (Magalhães, Jacobi, 2008).

Control or eradication with rotenone to remove non-native species that can compete with native fishes, selective removal of non-native species using electrofishing and netting, elimination of non-native grasses at the margins may remove protection/feeding sites and reduce abundance of vegetation-dependent forms (*i.e*. non-native juveniles), native species reintroduction to the reclaimed habitats followed by habitat restoration activities such as flow restoration, introduction of woody debris or riparian vegetation rehabilitation (Kennard *et al*., 2005; Rayner, Creese, 2006; Casatti *et al*., 2009) are all measures that will only be effective if the prevention policies are put into practice.

Studies about direct effects of non-native poeciliids on native competitor species are needed, with special emphasis on hybridization and adult competition for shelter, food, and reproductive sites (Alves *et al*., 2007). When non-native poeciliids of the genera *Poecilia* and *Xiphophorus* co-occur, negative impacts are observed on native communities of small water bodies (Arthington *et al*., 1983; Englund, 1999; Goren, Galil, 2005; Stockwell, Henkanaththegedara, 2011), and this situation already occurs in a headwater creek located in southern Brazil (Vieira, Shibatta, 2007). The use of artificial refuges could also promote coexistence between non-native poeciliids and native species (Westhoff *et al*., 2013; Magellan, García-Berthou, 2016).

Finally, careful management of poeciliid introductions can help Brazil avoid the catastrophic invasions that have occurred elsewhere (Arthington *et al*., 1983; Englund, 1999; Goren, Galil, 2005; Stockwell, Henkanaththegedara, 2011). The establishment of non-native poeciliids in Brazilian headwater creeks is the successful combination of appeal to humans (popular and inexpensive aquarium fish), propagule pressure (multiple introduction events), invasiveness (extended breeding season, small broods of large embryos, female-biased sex ratio, sperm storage, independence of external environmental conditions to reproduce), and invasibility (tropical region, low native species richness, absence of top predators). These fish already have and will continue to have significant impacts in the native biological communities of headwater creek ecosystems if the recommendations above, in particular those concerning cessation of propagule pressure, are not implemented.

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